

SUPPLEMENTAL MATERIAL

Disk diffusion assay

The agar disc diffusion method was used as preliminary assay for testing the antibacterial effect of the ICs against *S. aureus* USA300 and its derivative strains. A total of 0.5 mL from a 10^7 CFU/mL bacterial suspension was spread on the surface of TSAYE plates. Sterile filter paper disks (Whatman Grade 1, 6 mm diameter) were individually impregnated with 1, 2.5, and 30 μ L of carvacrol, citral, and (+)-limonene oxide, respectively, dissolved in 3% absolute ethanol (Sigma-Aldrich), and then placed on the previously inoculated agar plates. These volumes were previously determined to produce an inhibition halo measurable (data not shown). Plates were kept at 4 °C for 2 h to allow for the diffusion of ICs, and then incubated at 37 °C for 18–24 h. The diameters of the resulting zones of inhibition were measured with a Vernier digital micrometre (accuracy: 0.01 mm) including the diameter of the paper disk. Disks impregnated with 3% ethanol caused no inhibition halos.

Table S1. Zones of growth inhibition (mm) showing antibacterial activity for carvacrol (1 μ L), citral (2.5 μ L), and (+)-limonene oxide (30 μ L) against *Staphylococcus aureus* USA300 wild type and its derivative strains isolated: CAR, CIT, and OXLIM. Each value represents the mean diameter of the inhibition halo \pm standard deviation of the six colonies isolated by each IC. Increases in MIC with regard to WT are shaded.

.	WT	CAR	CIT	OXLIM
Carvacrol	20.49 \pm 0.31	14.79 \pm 0.61*	17.12 \pm 0.60*	16.21 \pm 0.49*
Citral	25.73 \pm 0.37	26.01 \pm 0.49 ^{ns}	19.14 \pm 0.21*	19.77 \pm 0.53*
(+)-Limonene oxide	16.04 \pm 0.54	16.12 \pm 0.38 ^{ns}	14.34 \pm 0.67*	11.18 \pm 0.29*

^{ns} not significantly different from wild type ($p > 0.05$).

* significantly different from wild type ($p \leq 0.05$).

Table S2. Primers used for verification.

Verification	Sequence F (5' → 3')	Sequence R (5' → 3')
Deleted 43 kb region in CAR	CTGACAATAGTGCCAAAGCCG	TGGGGTGTACATTCCACACA
	GGGACCCATTAGGGACTCCA	GGCCGAGTACAGGTGTTGA
Flanking regions of 43 kb deletion in CAR	CTGACAATAGTGCCAAAGCCG	GGCCGAGTACAGGTGTTGA
<i>aroC</i> SNP in CIT	TCGCATTGCTTGTGCGATAC	TTGCAGTCGGTGCCTTATGT
<i>rpoB</i> SNP in OXLIM	TGGTGCAGAAGTAAAAGATGGAGA	TCAAGAACGATAACGCCAGC

Table S3. Large deletion of *Staphylococcus aureus* CAR derivative strain.

Locus tag	Gene	Product	Locus tag	Gene	Product
SAUSA300_RS10505	<i>hlb-1</i>	phosphodiesterase	SAUSA300_RS10680		hypothetical protein
SAUSA300_RS10510		hypothetical protein	SAUSA300_RS10685		DUF1381 domain-containing protein
SAUSA300_RS10515		hypothetical protein	SAUSA300_RS10690		hypothetical protein
SAUSA300_RS10520		hypothetical protein	SAUSA300_RS10695	<i>dut</i>	dUTP pyrophosphatase
SAUSA300_RS10525	<i>scn</i>	staphylococcal complement inhibitor	SAUSA300_RS10700		hypothetical protein
SAUSA300_RS10530	<i>chp</i>	chemotaxis inhibitory protein	SAUSA300_RS10705		DUF1024 domain-containing protein
SAUSA300_RS10535		peptidoglycan hydrolase	SAUSA300_RS10710		hypothetical protein
SAUSA300_RS10540	<i>sak</i>	staphylokinase	SAUSA300_RS10715		hypothetical protein
SAUSA300_RS10545	<i>ami</i>	amidase	SAUSA300_RS10720		hypothetical protein
SAUSA300_RS10550		phage holin	SAUSA300_RS10725	<i>rusA</i>	Holliday junction DNA helicase
SAUSA300_RS10555		hypothetical protein	SAUSA300_RS10730		hypothetical protein
SAUSA300_RS10565		DUF2951 domain-containing protein	SAUSA300_RS10735	<i>dnaD</i> 2	replication protein DnaD
SAUSA300_RS10570		hypothetical protein	SAUSA300_RS10740	<i>ssb2</i>	single-stranded DNA-binding protein
SAUSA300_RS10575		hypothetical protein	SAUSA300_RS10745		MBL fold metallo-hydrolase
SAUSA300_RS10580		hypothetical protein	SAUSA300_RS10750	<i>recT</i>	recombinase
SAUSA300_RS10585		phage tail protein	SAUSA300_RS10755		ATPase
SAUSA300_RS10590		phage tail tape measure protein	SAUSA300_RS10760		DUF1108 domain-containing protein
SAUSA300_RS10600		hypothetical protein	SAUSA300_RS10765		hypothetical protein
SAUSA300_RS10605		phage tail protein	SAUSA300_RS10770		DUF1270 domain-containing protein
SAUSA300_RS10610		hypothetical protein	SAUSA300_RS10775		DUF771 domain-containing protein
SAUSA300_RS10615		hypothetical protein	SAUSA300_RS10780		hypothetical protein
SAUSA300_RS10620		phage head-tail adapter protein	SAUSA300_RS10785		oxidoreductase
SAUSA300_RS10625		phage head-tail adapter protein	SAUSA300_RS10790		hypothetical protein
SAUSA300_RS10630		hypothetical protein	SAUSA300_RS10795		DUF2829 domain-containing protein
SAUSA300_RS10635		phage major capsid protein	SAUSA300_RS10800		XRE family transcriptional regulator
SAUSA300_RS10640		Clp protease ClpP	SAUSA300_RS10805		transcriptional regulator
SAUSA300_RS10645		phage portal protein	SAUSA300_RS10810		XRE family transcriptional regulator
SAUSA300_RS10650		terminase large subunit	SAUSA300_RS10815		ATP-dependent helicase
SAUSA300_RS10655		hypothetical protein	SAUSA300_RS10820		hypothetical protein
SAUSA300_RS10660		HNH endonuclease	SAUSA300_RS10825		hypothetical protein
SAUSA300_RS10665		hypothetical protein	SAUSA300_RS10830		toxin MazF
SAUSA300_RS10670		hypothetical protein	SAUSA300_RS10835	<i>int3</i>	site-specific integrase
SAUSA300_RS10675		transcriptional activator RinB			